Appl Microbiol Biotechnol (2004) 63:653–658
DOI 10.1007/s00253-003-1400-x

ORIGINAL PAPER

T. C. Ezeji · N. Qureshi · H. P. Blaschek

Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping

Received: 24 March 2003 / Revised: 6 June 2003 / Accepted: 20 June 2003 / Published online: 9 August 2003

Abstract Acetone butanol ethanol (ABE) was produced in an integrated fed-batch fermentation-gas stripping product-recovery system using *Clostridium beijerinckii* BA101, with H_2 and CO_2 as the carrier gases. This technique was applied in order to eliminate the substrate and product inhibition that normally restricts ABE production and sugar utilization to less than 20 g l^{-1} and 60 g l^{-1}, respectively. In the integrated fed-batch fermentation and product recovery system, solvent productivities were improved to 400% of the control batch fermentation productivities. In a control batch reactor, the culture used 45.4 g glucose l^{-1} and produced 17.6 g total solvents l^{-1} (yield 0.39 g g^{-1}, productivity 0.29 g l^{-1} h^{-1}). Using the integrated fermentation-gas stripping product-recovery system with CO_2 and H_2 as carrier gases, we carried out fed-batch fermentation experiments and measured various characteristics of the fermentation, including ABE production, selectivity, yield and productivity. The fed-batch reactor was operated for 201 h. At the end of the fermentation, an unusually high concentration of total acids (8.5 g l^{-1}) was observed. A total of 500 g glucose was used to produce 232.8 g solvents (77.7 g acetone, 151.7 g butanol, 3.4 g ethanol) in 1 l culture broth. The average solvent yield and productivity were 0.47 g g^{-1} and 1.16 g l^{-1} h^{-1}, respectively.

N. Qureshi (✉)
Fermentation/Biotechnology Research,
United States Department of Agriculture,
National Center for Agricultural Utilization Research,
1815 N University Street, Peoria, IL 61604, USA
e-mail: QURESHIN@ncaur.usda.gov
Tel.: +1-309-6816567
Fax: +1-309-6816427

T. C. Ezeji · N. Qureshi · H. P. Blaschek
Biotechnology and Bioengineering Group,
Department of Food Science and Human Nutrition,
University of Illinois,
1207 W Gregory Drive, Urbana, IL 61801, USA

Introduction

Before the 1950s, clostridial acetone butanol ethanol (ABE) fermentation ranked second to ethanol in its importance and scale of production; but this declined due to increasing substrate costs and the availability of the much cheaper, petrochemically derived butanol (Dürre 1998). ABE production via clostridia has been widely studied, especially following the oil crisis in 1973. This led to renewed interest in ABE fermentation from renewable resources (cane molasses, corn, wood hydrolysate, etc.) and investigation into product recovery and the genetics of *Clostridium* species. The possible re-introduction of large-scale ABE fermentation appears increasingly feasible, owing in part to recent advances in our understanding of the genetics and physiology of solvent production by these microorganisms and in part to economic and environmental considerations (Lee et al. 2001; Qureshi and Blaschek 2001a, 2001b; Jesse et al. 2002). However, the cost of butanol recovery still remains high, because its concentration in the fermentation broth is low. The usual concentration of total solvents (ABE) in the fermentation broth is 20 g l^{-1}, of which butanol is only about 13 g l^{-1} (Qureshi and Blaschek 2001a). This limits the use of dilute sugar solutions in batch reactors (to 60 g l^{-1}). Butanol, which is a good fuel additive (reduces exhaust smoke) is highly toxic to the cells of *Clostridium acetobutylicum* and *C. beijerinckii* that produce it. For this reason, the low concentration of butanol in the fermentation broth requires significantly high energy for recovery.

Studies on butanol removal from the fermentation broth using distillation showed that, as the concentration of butanol increased from 10 g l^{-1} to 40 g l^{-1}, the ratio of oil used for fuel to 100% recovered butanol decreased from 1.5 t t^{-1} to 0.25 t t^{-1} (Phillips and Humphrey 1983). This suggests that significant energy savings can be achieved if the concentration of butanol in the fermentation broth is increased. In order to save on fuel oil (for butanol recovery) and economize butanol production, numerous techniques for ABE recovery have been

investigated and integrated with ABE production by fermentation. These techniques include adsorption, liquid–liquid extraction, gas stripping, perstraction, reverse osmosis and pervaporation. Among these techniques, gas stripping has numerous advantages (Ezeji et al. 2003) and is viewed as one of the more economic techniques (Groot et al. 1992). For this reason, gas stripping was investigated in *C. beijerinckii* BA101 batch fermentations. In a concentrated-substrate batch fermentation, 75.9 g ABE l^{-1} was produced from 161.7 g glucose l^{-1}. The substrate concentration used in this fermentation was over 2.5 times that used in batch fermentation. Additionally, a high product concentration was obtained, demonstrating the superiority of this product-removal system in integrated ABE fermentations. However, the problem associated with this system was that a sugar concentration higher than162 g l^{-1} could not be used because of substrate inhibition. A substrate concentration of 161.7 g l^{-1} resulted in a lag phase of approximately 40 h.

Fed-batch fermentation is an industrial technique where the reactor is started with a relatively low substrate concentration (to reduce substrate inhibition) and a low volume. As the substrate is consumed, it is replaced by adding a concentrated substrate solution at a low rate while keeping the substrate concentration in the reactor below the toxic level (Qureshi et al. 2001; Qureshi and Maddox 1990; Qureshi and Blaschek 2000). Fed-batch fermentation is advantageous in cases where an initial high substrate concentration is toxic to the culture. However, when applied to systems such as butanol, which is toxic, a product-removal technique should be applied in combination with the fed-batch fermentation. By feeding the reactor at a slow and controlled rate, substrate toxicity can be kept below inhibitory levels, while the product-removal technique can be applied simultaneously to remove butanol toxicity. Thus, application of these two engineering techniques solves two toxicity problems: one for substrate inhibition and another for butanol inhibition.

Since our batch reactor could not utilize more than 161.7 g glucose l^{-1}, a fed-batch fermentation product-recovery system was designed with a view to eliminate substrate and product inhibition and achieve a high productivity by eliminating the lag phase due to substrate inhibition. In addition, we attempted to use more sugar (per liter culture volume) and produce more solvents (ABE) per liter culture volume. The product inhibition was eliminated by removing ABE by a gas-stripping technique.

Materials and methods

Organism, culture maintenance and fermentation conditions

The organism used was a hyper-butanol-producing mutant designated *C. beijerinckii* BA101. It was generated using *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, together with selective enrichment of the non-metabolizable glucose analogue, 2-deoxyglucose (Annous and Blaschek 1991). A laboratory stock of *C. beijerinckii* BA101 was routinely maintained as spore suspensions in sterile double-distilled water at 4 °C. *C. beijerinckii* BA101 spores (200 µl) were heat-shocked for 10 min at 80 °C, followed by cooling in ice-cold water. It was inoculated into 20 ml tryptone/glucose/yeast extract (TGY) medium (in 50 ml screw-capped Pyrex bottle) and was incubated anaerobically for 15–16 h at 36±1 °C.

Batch fermentation

For comparison purposes, a 2-l bioreactor (New Brunswick Scientific Co., New Brunswick, N.J.) was used throughout the study. A fermentor containing glucose (60–100 g l^{-1} in 1.6-l reaction volume) and yeast extract (1 g l^{-1}) was sterilized at 121 °C for 15 min. On cooling to 35 °C under an O_2-free N_2 atmosphere, filter-sterilized P2 stock solutions were added (containing, per liter: 50 g KH_2PO_4, 50 g K_2HPO_4, 220 g ammonium acetate as buffer solution, 0.1 g para-amino-benzoic acid, 0.1 g thiamin, 0.001 g biotin as vitamin solution, 20 g $MgSO_4{\cdot}7H_2O$, 1 g $MnSO_4{\cdot}H_2O$, 1 g $FeSO_4{\cdot}7H_2O$, 1 g NaCl as mineral solution; Qureshi and Blaschek 1999), followed by inoculation of the bioreactor with 5% (v/v) highly motile cells of *C. beijerinckii* BA101 (16–18 h culture). O_2-free N_2 gas was swept over the headspace of the fermentor until the culture initiated production of its own gases (CO_2, H_2). Samples were withdrawn at intervals for analysis. Being a control experiment, gas stripping was not applied in this reactor.

Fed-batch fermentation and gas stripping

A 2-l bioreactor containing P2 medium as in case of the batch fermentation (glucose concentration 99.9 g l^{-1}) was inoculated with 5% (v/v) highly motile cells of *C. beijerinckii* BA101. The fermentation was allowed to proceed in batch mode for 22 h (ABE concentration approximately 5 g l^{-1}), after which gas stripping was applied, using O_2-free N_2 gas. Prior to the gas-stripping experiment, the condenser and gas-circulation line were flushed with O_2-free N_2 gas, to make it anaerobic. This was followed by passing enough fermentation gases (CO_2, H_2) through the line. Gas stripping was initiated (after 15 h of fermentation) by recycling CO_2 and H_2 through the system (6,000 ml min^{-1}), using a twin-head peristaltic pump. The cooling machine (GeneLine) was obtained from Beckman Instruments (Palo Alto, Calif.). The ABE vapors were cooled in a condenser (62×600 mm, cooling coil surface area 1,292 cm^2) to −2 °C, using ethylene glycol (50% v/v) circulated at a flow rate of 600 ml min^{-1} through the condenser. O_2-free distilled water was added to the reactor at intervals, to maintain a constant liquid level inside the reactor (compensating for water loss due to gas stripping). A schematic diagram of the integrated reactor set up is shown in Fig. 1. There was no mechanical agitation or pH control; and the temperature was controlled at 33–35 °C during the entire process. Antifoam 204 (Sigma Chemicals, St. Louis, Mo.) was used as an antifoam agent and was added manually. Samples were aseptically withdrawn at intervals for glucose, ABE and optical density analysis. Based on the sugar and optical density results, concentrated glucose solution (500 g l^{-1}) and nutrients were added to the reactor at intervals, to replace the utilized glucose and nutrients. The feed medium was kept anaerobic using O_2-free N_2 gas. During fermentation, the pH was not controlled as there is normally a pH-breakpoint associated with the transition to solventogenesis.

To examine why the fed-batch fermentation stopped, we centrifuged the fermentation broth at 10,000 rpm for 5 min and collected the supernatant. Glucose was added to the supernatant to make the final concentration up to 60 g l^{-1}. Then, the supernatant was divided into four equal volumes (100 ml). To each of the first two volumes (A, B) 1 g yeast extract l^{-1} was added, while no nutrients were added to the other two volumes (C, D). The five screw-capped bottles containing supernatant and control (60 g glucose l^{-1}, P2 medium) were sterilized at 121 °C for 15 min. On cooling to room temperature, filter-sterilized P2 medium stock solutions were added to each of the first two volumes (A, B) with

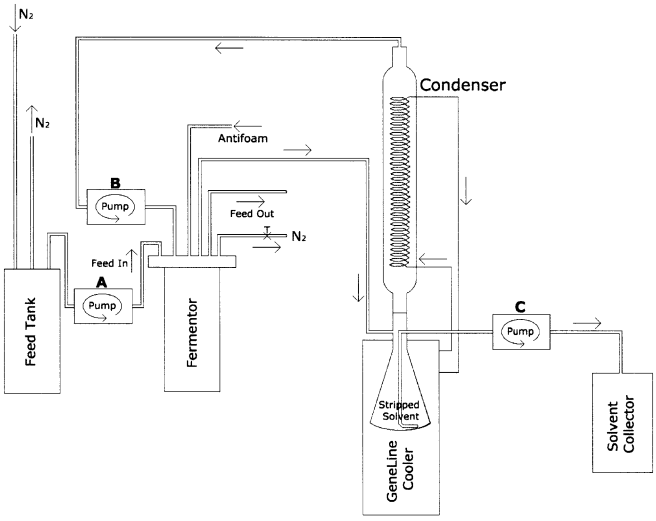

Fig. 1 Schematic diagram of fed-batch acetone butanol ethanol (ABE) production by *Clostridium beijerinckii* BA101 and recovery by gas stripping. *A*, *B* and *C* are pumps for feed, gas recycling and product removal, respectively

1 g yeast extract l^{-1} (pH 5.8), while no nutrients were added to the other two volumes (C, D). This was followed by transfer to an anaerobic chamber (Coy Laboratories Products, Ann Arbor, Mich.), where they were kept for 24 h prior to inoculation with 5 ml freshly grown cells (in TGY medium). They were incubated at 36±1 °C.

Analytical procedures

Cell concentration was estimated by optical density and a cell dry weight method, using a predetermined correlation between optical density at 540 nm and cell dry weight. ABE and acids (acetic, butyric) were measured using a gas chromatograph (model 6890; Hewlett-Packard, Avondale, Pa.) equipped with a 1,830×2 mm glass column (10% CW-20M, 0.01% H_3PO_4, support 80/100 Chromosorb WAW) and a flame Ionization detector. Productivity was calculated as total ABE produced (grams per liter culture volume) divided by fermentation time (h). Yield was defined as total grams ABE produced per total grams glucose utilized. Selectivity was calculated as α=[y/(1−y)]/[x/(1−x)], where x and y are weight fractions of acetone, butanol and ethanol in fermentation broth and condensate, respectively. Selectivity is a measure of the preferential removal of solvents over other components present in the mixture, such as water. The rate of glucose utilization was defined as grams glucose utilized per liter culture volume for a given time interval. The rate of solvent production was defined as total grams ABE produced during a particular time interval.

Glucose concentration was determined using a hexokinase and glucose-6-phosphate dehydrogenase coupled enzymatic assay (Sigma Chemicals). The fermentation broth was centrifuged (microfuge centrifuge) at 14,000 rpm for 3 min at 4 °C. A portion of the supernatant (10 µl) was mixed with glucose (HK) 20 reagent (1.0 ml) and incubated at room temperature for 5 min. Standard solutions of anhydrous D-glucose containing 1–5 g glucose l^{-1} distilled water were prepared and 10 µl of each standard solution were mixed with glucose (HK) 20 reagent (1.0 ml) and incubated at room temperature for 5 min. A blank (10 µl deionized water) was incubated with the reagent and was used for zero adjustment of the spectrophotometer. After 5 min, the absorbance was measured at 340 nm, using a Beckman DU 640 spectrophotometer and the glucose content in the sample was computed by least squares linear regression, using a standard curve.

Results

A control batch fermentation experiment was run with 59.9 g initial glucose l^{-1} in P2 medium using *C. beijerinckii* BA101. Over the course of 60 h, the culture produced 17.6 g total solvents l^{-1}, with a solvent yield of 0.39 (Table 1). At the end of fermentation, 14.5 g glucose l^{-1} remained unused because of solvent inhibition. A previous integrated batch experiment conducted with 161.7 g glucose l^{-1} resulted in the production of 75.9 g ABE l^{-1} (Ezeji et al. 2003). The culture experienced a long lag phase (38–40 h, which is significant for a 126 h fermentation), resulting in a reduced productivity. *C. beijerinckii* BA101 is inhibited above a glucose concentration of 100 g l^{-1}.

Based on the above criteria, feeding concentrated glucose solution (500 g l^{-1}) during fermentation with simultaneous product removal (gas stripping) was adapted

Table 1 Production of acetone butanol ethanol (ABE) in a control fermentation and an integrated fed-batch fermentation coupled with gas stripping for product-recovery, using *Clostridium beijerinckii* BA101

Parameter	Control (60 g glucose l^{-1})	Integrated fed-batch fermentation
Acetone (g l^{-1})	5.2	77.7
Butanol (g l^{-1})	11.9	151.7
Ethanol (g l^{-1})	0.5	3.4
Total ABE (g l^{-1})	17.6[a]	232.8
ABE yield (g g^{-1})	0.39	0.47
ABE productivity (g l^{-1} h^{-1})	0.29	1.16
Acetic acid (g l^{-1})	0.5	4.3
Butyric acid (g l^{-1})	0.4	4.2
Total acids (g l^{-1})	0.91	8.5
Initial glucose (g l^{-1})	59.9[a]	100.0
Final glucose (g l^{-1})	14.5	26.1
Glucose utilized (g l^{-1})	45.4	500.1
Glucose utilization (%)	75.9	95.1
Glucose utilization rate (g l^{-1} h^{-1})	0.76	2.49
Maximum cell concentration (g l^{-1})	3.2	15.0
Cell yield (g g^{-1})	0.07	0.03
Fermentation time (h)	60	201
Glucose concentration in feed (g l^{-1})	–	500.0

[a] Data taken from Ezeji et al. (2003)

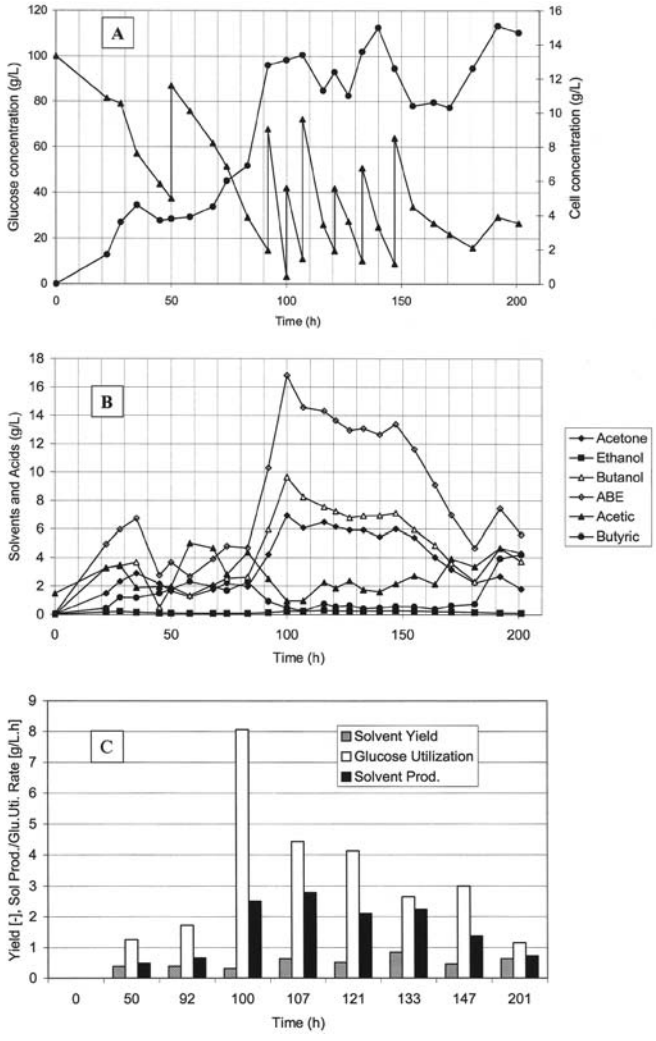

Fig. 2A–C Production of ABE in a fed-batch gas-stripping system using *C. beijerinckii* BA101. **A** Glucose (*triangles*) and cell concentration (*circles*), **B** solvents and acids, **C** productivity, yield and glucose utilization

to increase productivity. Furthermore, the application of concentrated substrates would reduce the size of the fermentation equipment and the volume of the waste-disposal streams. Glucose and P2 nutrients (each time: equal to half of that added to the fresh medium) were added during fermentation at 50, 92, 100, 107, 121, 133 and 147 h and at 50, 83, 116, 127, 147 and 171 h, respectively (Fig. 2A, B). There was no bleed of the fermentation broth from the fermentor. The addition of glucose and nutrient solutions depended upon the residual glucose and the cell concentration in the fermentation broth. The maximum concentration of glucose did not exceed 90 g l^{-1}. The cell concentration increased with time to a maximum of 15 g l^{-1} at the end of fermentation (Fig. 2A). Figure 2B shows the concentration of acetone, butanol and ethanol present in the fermentation broth during gas stripping; and the maximum amount of ABE recorded was 16.8 g l^{-1}, which is within the tolerance level of the culture. An important part of this experiment is that adequate amounts of nutrients should be added intermittently for a successful fermentation.

Solvent (ABE) yield, glucose utilization rates and solvent production rates at various times are shown in Fig. 2C. The yield was often found to be high because of utilization of acids produced in the fermentation and utilization of sodium acetate (buffer component) for solvent production. At 100 h, the glucose utilization rate was high due to solvent production and cell growth, which coincided with a high concentration of ABE in the reactor. The rates of glucose consumption and solvent production during the 0–92 h of fermentation were relatively low because of the initial low cell concentration. The rate of glucose utilization increased significantly between 92–100 h of fermentation (Fig. 2C) and the cell concentration increased from 6.9 g l^{-1} (after 83 h) to 13.1 g l^{-1} (after 100 h), as shown in Fig. 2A. A maximum solvent production rate (2.79 g l^{-1} h^{-1}) was recorded at 107 h of fermentation. In the present studies, the fed-batch fermentation product-recovery system was run for 201 h, at which point it terminated. In this product-recovery system, the maximum ABE produced, maximum cell concentration, overall yield and solvent productivity were 232.8 g l^{-1} (including losses), 15.0 g l^{-1}, 0.47 g l^{-1} h^{-1} and 1.16 g l^{-1} h^{-1}, respectively, as shown in Table 1. After 181 h of fermentation, the culture started experiencing difficulties switching from acidogenesis to solventogenesis and the pH suddenly dropped from 6.6 to 5.4. The fermentation stopped after 201 h when the glucose and total acid levels were 26.1 g l^{-1} and 8.5 g l^{-1}, respectively, as shown in Fig. 2A, B.

In order to identify the cause of cessation of fermentation, experiments were conducted in bottles. Cell growth was vigorous in the control but there was no growth in C and D, while A and B recorded poor growth, as shown in Table 2. When viewed under the microscope, the cells from A and B were 3–4 times more elongated and less motile in comparison with the control.

Table 2 Cell growth of *C. beijerinckii* BA101 in control and spent fermentation broths

Time (h)	Control (g l^{-1})	Spent broth + nutrients in bottles A, B (g l^{-1})	Spent broth in bottles C, D (g l^{-1})
0	0.002	0.002	0.002
24	1.80	0.28	0.002
36	2.36	0.24	0.000
48	2.83	0.13	0.000
60	2.40	0.14	0.000

ABE was selectively removed during the fermentation process. During the fed-batch fermentation (22–201 h), butanol selectivities were 10.34–22.07. Acetone and ethanol selectivities ranged over 6.69–12.72 and 4.45–11.16, respectively. As published previously (Ezeji at al. 2003), the results did not show any direct relationship between fermentation broth ABE concentration and each component′s respective selectivity. Previous experiments showed that solvent selectivities were also affected by the cell concentration in the fermentation broth. Acetic and butyric acids were not detected in the condensate.

Discussion

Examination of the results (Fig. 2) showed that the rate of solvent production decreased significantly after 133 h, even though cell concentration (Fig. 2A) was relatively stable (and even increased after 171 h). The presence of inactive or dead cells, low water activity, accumulation of inhibitory macromolecules (like polysaccharides) and deficiency of nutrients were earlier given as possible reasons for the decreased productivities during a pervaporation experiment with *C. acetobutylicum* (Qureshi et al. 2001). Furthermore, in a *C. acetobutylicum* reactor (Qureshi et al. 1988), four different types of cells were found: dead cells, growing cells, solvent-producing cells and cells requiring maintenance energy (neither growing nor producing solvents). Dead cells or spores neither grow nor produce solvents, resulting in low specific productivity.

Several reasons may provide the cause for the cessation of the fed-batch fermentation, including lack of nutrients, diffusion of O_2 through the connecting tubes during nutrient and glucose solution addition, toxicity of minerals (from P2 medium), low water activity and/or accumulation of undetermined fermentation products (macromolecules). It should be noted that there was no bleed throughout the experimental period. However, experiments conducted in bottles (A, B, C, D) suggested that the fermentation terminated because of inhibition, either by unknown fermentation products or a lowered water activity, or possibly the culture degenerated due to toxicity. Culture degeneration is a feature usually associated with genetic change and takes place over a longer period of time, particularly during continuous cultivation. Detailed studies of *C. acetobutylicum* degeneration during continuous fermentation showed that a population of non-solventogenic *Clostridia* appears, which coexists for some time with the solventogenic culture and gradually takes over (Woolley and Morris 1990). In our experiment, there was a sudden cessation of ABE production due to an apparent failure of a "switch" from acidogenic to solventogenic culture, a phenomenon known as "acid crash", which occasionally occurs in pH-uncontrolled batch fermentations. It is proposed that the "acid crash" occurs when the acid concentration in the fermentation broth exceeds the maximum tolerable limit, causing cessation of glucose uptake and rapid termination of solventogenesis after the switch has occurred (Maddox et al. 2000). In experiments where "acid crash" occurred, the pH value was less than 5.0 (Maddox et al. 2000). Therefore, "acid crash" may not be the real cause for the cessation of this fed-batch fermentation but may have contributed to the premature termination of the fermentation because of the high amount of butyric acid (Fig. 2B), which is more toxic than acetic acid. Geng and Park (1994) observed that cells of *C. acetobutylicum* B18 became long-rod-shaped during a fermentation product-recovery process after 56 h of operation and there was cell autolysis and cell death.

It should be noted that acids produced during the recovery experiment were utilized for solvent production. This may have resulted in improved productivity and yield. Furthermore, the gas-stripping procedure does not remove acids from the fermentor. Rather, it enhances total conversion of acetic and butyric acids to solvents by the culture. Glucose utilization up to 100% was recorded in the two integrated processes (Ezeji et al. 2003), compared with 75.9% in the non-integrated process. It should be noted that a high productivity of 1.16 g l^{-1} h^{-1} was achieved in the integrated experiment, as compared with 0.29 g l^{-1} h^{-1} in the control experiment. Glucose utilization was over 95% of that fed to the system (526.1 g l^{-1}). From the point of view of eliminating substrate and product inhibition, these studies were successful. One of the reasons for the high yield was that a lower amount of glucose was used for cell growth (0.03 g g^{-1}) as opposed to 0.07 g g^{-1} in the control experiment. Further, we are investigating the effect of bleed on the sustainability of a continuous fermentation-recovery system. The fed-batch fermentation and product-recovery experiment was not repeated. However, it is believed that, under similar fermentation conditions (including culture stability), up to 500±25 g glucose l^{-1} can be fermented to produce ABE.

In conclusion, ABE was produced in a fed-batch fermentor integrated with a gas-stripping product-removal system. By combining the fed-batch reactor with product recovery, inhibition for both substrate and product was eliminated. During the gas-stripping process, gases were recycled at 6,000 ml min^{-1} through the system to remove acetone and butanol from the fermentation broths. It was found that the process kept the ABE concentration below the level toxic to the culture without impeding ABE production. Acetic and butyric acids were not removed from the fermentor by the gas-stripping process. Compared with the non-integrated batch reactor (17.6 g total solvent l^{-1}), 13 times more solvents (232.8 g total solvent l^{-1}) were produced in this system. The average solvent yield (0.47) was found to be higher than in the non-integrated batch reactor (0.39); and the productivity (1.16 g l^{-1} h^{-1}) was 4 times higher than the batch reactor (0.29 g l^{-1} h^{-1}). Furthermore, in the fed-batch integrated system, the glucose utilization rate (2.49 g l^{-1} h^{-1}) was 3.3 times higher than the batch reactor (0.76 g l^{-1} h^{-1}). Accumulation of minerals, macromolecules (polysaccharides) and low water activity may have terminated the

fermentation, although this was not further investigated. Therefore, during a long-term fed-batch fermentation using *C. beijerinckii* BA101 culture with an integrated gas-stripping product-removal system, bleeding of the bioreactor to get rid of these types of macromolecules may be recommended.

Acknowledgements This work was supported by the Illinois Council on Food and Agricultural Research (C-FAR), grant numbers CFAR 0II-042-1 and IDA CF 01E-35-1. N.Q. would like to thank Michael A. Cotta (United States Department of Agriculture, National Center for Agricultural Utilization Research, Peoria, Ill.) for his contribution to this work.

References

Annous BA, Blaschek HP (1991) Isolation and characterization of *Clostridium acetobutylicum* mutants with enhanced amylolytic activity. Appl Environ Microbiol 57:2544–2548

Dürre P (1998) New insights and novel developments in clostridial acetone/butanol/isopropanol fermentation. Appl Microbiol Biotechnol 49:639–648

Ezeji TC, Qureshi N, Blaschek HP (2003) Production of acetone butanol ethanol by *Clostridium beijerinckii* BA101 and in-situ recovery by gas stripping. World J Microbiol Biotechnol (in press)

Geng Q, Park C-H (1994) Pervaporative butanol fermentation by *Clostridium acetobutylicum* B18. Biotechnol Bioeng 43:978–986

Groot WJ, Lans van der RGJM, Luyben KCAM (1992) Technologies for butanol recovery integrated with fermentations. Process Biochem 27: 61–75

Jesse TW, Ezeji TC, Qureshi N, Blaschek HP (2002) Production of butanol from starch based packing-peanuts and agricultural waste. J Ind Microbiol Biotechnol 29:117–123

Lee J, Mitchell WJ, Blaschek HP (2001) Glucose uptake in *Clostridium beijerinckii* NCIMB 8052 and the solvent-hyper-producing mutant BA101. Appl Environ Microbiol 67:5025–5031

Maddox IS, Steiner E, Hirsch S, Wessner S, Gutierrez NA, Gapes JR, Schuster KC (2000) The cause of "acid crash" and "acidogenic fermentations" during the batch acetone-butanol-ethanol (ABE-) fermentation process. J Mol Microbiol Biotechnol 2:95–100

Phillips JA, Humphrey AE (1983) An overview of process technology for the production of liquid fuels and chemical feedstocks via fermentation. In: Wise DL (ed) Organic chemicals from biomass. Benjamins/Cummings Publishing, Menlo Park, Calif., pp 249–304

Qureshi N, Blaschek HP (1999) Butanol recovery from model solutions/fermentation broth by pervaporation: evaluation of membrane performance. Biomass Bioenerg 17:175–184

Qureshi N, Blaschek HP (2000) Butanol production using *Clostridium beijerinckii* BA101 hyper-butanol producing mutant strain and recovery by pervaporation. Appl Biochem Biotechnol 84/86:225–235

Qureshi N, Blaschek HP (2001a) Recent advances in ABE fermentation: hyper-butanol producing *Clostridium beijerinckii* BA101. J Ind Microbiol Biotechnol 27:287–291

Qureshi N, Blaschek HP (2001b) ABE production from corn: a recent economic evaluation. J Ind Microbiol Biotechnol 27:292–297

Qureshi N, Maddox IS (1990) Application of fed-batch culture to the ABE fermentation process: relief of product inhibition by product separation techniques. Proc Australasian Chem Eng Conf 18:27–30

Qureshi N, Paterson AHJ, Maddox IS (1988) Model for continuous production of solvents from whey permeate in a packed bed reactor using cells of *Clostridium acetobutylicum* immobilized by adsorption onto bonechar. Appl Microbiol Biotechnol 29:323–328

Qureshi N, Meagher MM, Huang J, Hutkins RW (2001) Acetone butanol ethanol (ABE) recovery by pervaporation using silicate–silicone composite membrane from fed-batch reactor of *Clostridium acetobutylicum*. J Membr Sci 187:93–102

Woolly RC, Morris JG (1990) Stability of solvent production by *Clostridium acetobutylicum* in continuous culture: strain differences. J Appl Bacteriol 69:718–728